THE INFLUENCE OF CERTAIN DUCTLESS GLAND SUBSTANCES ON THE GROWTH OF PLANT TISSUES.[1]

ROBERT A. BUDINGTON.

That thyroid gland constituents and secretions contain substances which effect clear, and often far-reaching, influences on the metabolism and action of animal cells has long been recognized, both from observations of natural wild stock, and from experimental data. As a normally produced hormone, or as a substance introduced artificially, thyroid substances alter the norm of the metabolism of an adult tissue; in no less, but rather in more pronounced ways, thyroid tissue fed to growing embryos modifies their growth and differentiation.

Proof of the above has been furnished by numerous workers for vertebrates, especially for representatives of the mammalia and amphibia; and for insects and insect larvæ, as Northrop and Kunkel, respectively, have demonstrated. Protozoa also show a marked modification of their metabolic processes when they feed upon or absorb thyroid gland products, as indicated by the work of Nowikoff, Shumway, Budington and Harvey, and more recently by Chambers.

After noting that living substances in organisms of such widely different constitution and phylogenetic position seem distinctly susceptible to this hormonic material, the question arises,—Is thyroid substance a compound with special potencies over animal metabolism, or is all protoplasm amenable to its influence? Assuming a single origin of living material, has the wide divergence of the animal and plant kingdoms produced in the latter a type of protoplasm which may be immune to this glandular product, so foreign to plants in its place of synthesis?

This paper reports the morphological effects of thyroid constituents on the growth of the root-tips of the onion, *Allium*. To avoid seasonal eccentricities, the experiments were repeated

[1] From the Department of Zoölogy, Oberlin College.

three different years; and in this connection I am very glad to acknowledge much assistance from three senior students, Miss Helen F. Harvey, Miss Harriet M. Heeman and Miss Gladys Newman.

Methods and Results.

The experiments were carried out in mid-winter and early spring when the dormant period of onion bulbs is naturally terminating. Material was secured from the open market, from different dealers for successive trials; sound bulbs of medium size, suited to nicely cover the open tops of Naples staining jars of 45 mm. diameter, and 120 c.c. capacity, were selected; care was taken to discard any which already showed root growth.

The nutrient fluid used in the jars was Pfeffer's solution, made up according to the formula given in Duggar's "Plant Physiology." [1] Each experiment consisted of five or more groups of bulbs; to the nutrient fluid in jars supporting them was added desiccated thyroid gland (in tablet form put up by Parke, Davis and Co.) in the following amounts: Group I., 1 grain; Group II., 0.75 grain; Group III., 0.5 grain; Group IV., 0.25 grain; Group V. was the control, which grew on the nutrient solution alone. The solutions were stirred from time to time to secure homogeneity. Some experiments were carried out in a greenhouse affording approximately uniform temperature and humidity; but results obtained under these conditions did not differ from those conducted in the common laboratory environment.

A further detail may also be mentioned here; it was natural that solutions such as were used, containing organic substances, would gradually become infected with bacteria, no matter how sterile the ingredients at first. This feature of the procedure

[1] Pfeffer's solution as used:

Calcium nitrate	4 grams.
Potassium nitrate	1 "
Magnesium sulfate	1 "
Potassium dihydrogen sulfate	1 "
Potassium chloride	0.5 "
Iron chloride	trace.
Water dist	5 liters.

would be of more importance in case the solutions were employed for prolonged periods; but in this work the bacterial factor never seemed to interfere with entirely healthy root growth. Indeed, Curtis has shown that, in some instances at least, a heavy infection of bacteria and fungus filaments about root sprouts on cuttings seems to be favorable to their better growth. His experiments covered many weeks, often months; deleterious effects due to carbon dioxide production in the solution may follow such infection after extended periods; but the factor is negligible in experiments limited to ten-day or two-week periods.

The following photograph is essentially self-explanatory and may be taken as typical of many. Roots grew somewhat in

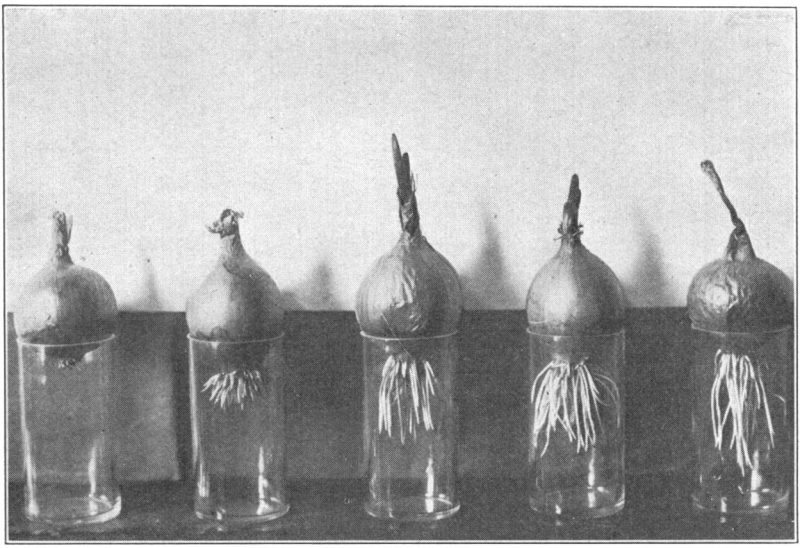

FIG. 1. Onion bulbs growing in Pfeffer's nutrient solution, in which is dissolved desiccated thyroid gland material, in parts of a grain indicated.

solutions of each different strength here mentioned; the very slight growth in the left hand bulb here figured would have continued to greater length after a time, but the proportionate lengths would have remained much as when the picture was taken. The apparent influence of the thyroid material is to retard growth rate.

Discussion.

The almost constant effect of abnormal amounts of thyroid tissues or extracts in the food or environment (or both) of developing animals has been to accelerate differentiation in premature ways. So too, one may interpret increased fission rate in Protozoa as indicating a similar effect, fission being a procedure characteristic of their attainment of adult physiological conditions. Such precocious metamorphosis of amphibian and insect larvæ is naturally coincident with small size.

The difficulties in estimating the effect of substances on plant tissues in terms of animal response and metabolism are obvious, and one should perhaps heistate to make any comparisons. However, all protoplasm obtains its raw materials through its permeable cell walls; and one may also go further and say that the root tips of bulbs, as they begin to grow, represent a kind of embryonic tissue with a minimum of specialization. In brief, the effect of thyroid substances on onion root-tips is to retard, or partially inhibit, size-growth; and this seems precisely the effect on embryonic animal tissue. In the latter, a hastening of physiologic and morphologic differentiation is also present; if the same be true in these root-tips, it naturally cannot be judged from external appearances, though one cannot deny that premature differentiation may be present. It is hoped to report on this point in a later communication.

Since the work of Marine and Lenhart, Morse, Swingle and others has made certain the earlier assumption that iodine is the most active principle in thyroid stuffs, a series of experiments in which only iodine was added to the nutrient solution was carried out. To make more significant and exact the comparison between such an experiment and those in which dessicated gland was used, KI was added in amounts based upon the accepted estimate that 0.01 c.c. of a saturated solution of KI furnishes the amount of iodine present in one grain of thyroid. Onion root-tips sprouting in media with this iodine content showed no growth-rate which was specifically different from that of controls growing in nutrient solution alone.

Further sets of experiments were carried out with pituitary gland tablets, using strengths of 2, 1.5, 1 and 0.5 grains in 120 c.c.

of the nutrient fluid; supra-renal gland tablets were also used, in strengths of 1, 0.75, 0.5 and 0.25 grains in 120 c.c. of nutrient fluid; but there was no uniform effect of these substances, the appearance being that these substances so used have no influence at all on this particular plant tissue.

SUMMARY.

1. Growth of root-tips of *Allium* is retarded by the presence in their fluid nutrient environment of thyroid gland material. Retardation is approximately in direct proportion to the amount of thyroid substance present.

2. The presence of thyroid materials in the nutrient fluid in which *Allium* is sprouting does not modify the growth of the early leaves.

3. Iodine, used as KI, in amounts equivalent to that in thyroid substances provoking marked modifications of growth, has no appreciable effect on growing root-tips.

4. Pituitary substances up to two grains of the desiccated gland, and supra-renal substances up to one grain of the desiccated gland in 120 c.c. of nutritive solution have no effect on the growing root-tips of *Allium*.

5. While no general conclusion can be based on experiments limited to a single form, the indication is that thyroid constituents may influence the rôle of protoplasmic action in cells other than those of animal tissues.

LITERATURE.

Budington, R. A., and Harvey, H. F.

'15 Division Rate in Ciliate Protozoa as Influenced by Thyroid Constituents. BIOL. BULL., 28, 304–314.

Chambers, Mary H.

'19 The Effect of some Food Hormones and Glandular Products on the Rate of Growth of *Paramecium caudatum*. BIOL. BULL., 36, 82–91.

Curtis, Otis F.

'18 Stimulation of Root Growth in Cuttings by Treatment with Chemical Compounds. Cornell Univ. Agr. Exp. Sta., Memoir 14, 71–138.

Kunkel, B. W.

'18 The Effects of the Ductless Glands on the Development of the Flesh Flies. Jour. Exper. Zoöl., 26, 255–264.

Marine, D., and Lenhart, C. H.

'09 Relation of Iodin to the Structure of Human Thyroids. Arch. Inter. Med., 4, 440–493.

Morse, Max

'14 The Effective Principle in Thyroid Accelerating Involution in Frog Larvæ. Jour. Biol. Chem., 19, 421.

Northrop, J. H.

'17 The Rôle of Yeast in the Nutrition of an Insect (*Drosophila*). Jour. Biol. Chem., 30, 181–187.

Nowikoff, M.

'08 Die Wirkung des Schilddrusenextrakts und einiger anderen organischen Stoffe auf Ciliaten. Arch. f. Protist., Bd. 11, 2.

Shumway, W.

'14 Effect of Thryoid on Division Rate of *Paramecium*. Jour. Exper. Zoöl. 17, 297–311.

'17 The Effects of Thyroid Diet upon *Paramecium*. Jour. Exper. Zoöl., 22, 529–563.

Swingle, W. W.

'18 Iodin as the Active Principle of the Thyroid Gland. Endocrinology, 2, 283–288.